

Effects of irregular antenatal care attendance in primiparas on the perinatal outcomes: a cross sectional study

Ahmed M. Abbas, MD,¹ Mona Rabeea, MSc,² Hamida A. Abdel Hafiz, MD,² Nadia H. Ahmed, MD²

Keywords: Antenatal care, primipara, perinatal outcome, maternal morbidity.

Abstract

Aim: Antenatal care (ANC) is a key strategy for reducing maternal and neonatal morbidity and mortality rates because adequate utilization of antenatal health care services is associated with improved maternal and neonatal health outcomes. The current study aims to identify the factors causing lack of regular attendance at ANC clinics in primiparas and to determine the effects of lack of antenatal care on perinatal outcomes.

Methods: A cross sectional study was carried out at Assiut Women's Health Hospital, Egypt between October 2015 and September 2016. Estimated sample size of 516 primiparous women were divided into two groups according to number of attendance to ANC clinics; women who came four visits or more were considered regular and those who came to less than four visits were considered irregular. Data was collected via a structured interview questionnaire that included personal data, obstetrical, data about current pregnancy and labor. The researcher interviewed the women within 24 hours postpartum.

Results: The majority of the study sample (74.8%) had regular ANC visits while only

(25.2%) had <4 antenatal visits. Financial problems (44.6%) or lack of awareness about the importance of ANC (20%) were the most common reasons for irregular ANC visits. The incidence of preeclampsia and eclampsia is significantly higher in the irregular attendants ($p=0.000$). Similarly, preterm birth, stillbirth and low birth weight were significantly more common among irregular attendants ($p=0.000$).

Conclusion: Women with irregular antenatal care attendance are much more prone to pregnancy complications such as preeclampsia, eclampsia and anemia besides higher adverse birth outcomes including preterm birth, low birth weight and stillbirth.

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Assiut, Egypt

²Department of Obstetric & Gynecology of Nursing, Faculty of Nursing, Assiut University, Egypt

Introduction

Antenatal care (ANC) is a preventive obstetric health program that aims at optimizing maternal-fetal outcome,

Please cite this paper as: Abbas AM, Rabeea M, Abdel Hafiz HA, Ahmed NH. Effects of irregular antenatal care attendance in primiparas on the perinatal outcomes: a cross sectional study. *Proc Obstet Gynecol.* 2017;7(2): Article 2 [11 p.]. Available from: <http://ir.uiowa.edu/pog/>. Free full text article.

Corresponding author: Ahmed M. Abbas, MD, Department of Obstetrics and Gynecology, Faculty of Medicine; Assiut University, Assiut, Egypt; Woman's Health Hospital, 71511, Assiut, Egypt, Cellular: +20 10033851833; Tel: +20 88 2414616; email: bmr90@hotmail.com

Financial Disclosure: The authors report no conflict of interest.

Copyright: © 2017 Abbas et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

through regular monitoring of pregnancy.¹ Antenatal visits are important for maternal and child health. Three main reasons have been advanced to explain the importance of ANC for pregnant women: promotion of health during pregnancy through counseling and educational activities, screening, identification and referral of women with risk factors; and health monitoring throughout pregnancy.²

Good quality ANC improves maternal health; decreases the chances of suffering from anemia, pregnancy induced hypertension, preterm labor and promotes positive pregnancy outcomes, including a reduced risk of low birth weight and preterm babies. ANC increases the use of a Skilled Birth Attendant during delivery and postnatal care because the visit can be an opportunity to educate women about the merits of skilled birth attendance. ANC visits provide an excellent opportunity to deliver education regarding the danger signs and symptoms during pregnancy, delivery and the postpartum period and to focus on birth spacing and family planning.³

The World Health Organization (WHO) recommends a minimum of four antenatal care visits. However, global estimates indicate that only about half of all pregnant women receive this recommended amount of care.⁴ In Egypt, the percentage of mothers who had regular antenatal care is 66%.⁴ According to the 2014 EDHS results, the percentage of mothers who had regular antenatal care prior to their last birth in Assiut city is 76.7%.¹

According to Andrew et al., in their 2014 study in Madang,⁵ factors influencing

attendance can be divided into three main categories: accessibility, attitudes to antenatal care, and interpersonal issues. Although women saw accessibility (distance and cost) as a barrier, those who lived close to health facilities and could easily afford antenatal care also demonstrated poor attendance. Attitudes were shaped by previous experiences of ANC, such as waiting times, quality of care, and perceptions of preventative care and medical interventions during pregnancy. Interpersonal factors included relationships with healthcare providers, pregnancy disclosure, and family conflict.⁵

Maternal complications and poor perinatal outcome are highly associated with non-utilization of antenatal and delivery care services. This leads to poorer outcomes in unbooked than booked patients.⁶ ANC services help pregnant women by identifying complications associated with the pregnancy or diseases that might adversely affect the pregnancy. Through antenatal visits, women benefit from various interventions, including counseling about healthy lifestyles, the provision of iron/folic acid supplements, and tetanus toxoid vaccinations.⁷

The aim of the study was to identify factors causing lack of regular attendance at antenatal care clinics in primiparas and to determine the effects of lack of ANC on perinatal outcomes.

Methods

Study type, setting and duration

The current study was a cross sectional study carried out at Assiut Women's

Health Hospital, Egypt between October 2015 and September 2016. It is the largest tertiary care hospital in Upper Egypt lying in Assiut city. The hospital provides its antenatal and labor services to nine governorates in Upper Egypt. The Assiut Medical School ethical review board approved the study. The non-interventional nature of the study and respect of patients' confidentiality were clear to the patient and their written consent to participate had been obtained.

Study population

All primiparous women who delivered during the study period were invited to participate in our study. The inclusion criteria included primiparous women who delivered whether vaginally or by cesarean section (CS) within the first postpartum 24 hours. We excluded those who had chronic medical illnesses and those who declined participation in the study.

Sample size

Sample size calculation was done using (Epi-info™, CDC, USA.2008) program using 80% power and level of significance was set at 5% ($P=0.05$) taking in consideration the population size; adjusted number of pregnant primiparas attended the labor ward in the preceded year before the study and the effect of poor antenatal care on pregnancy outcome established from the previous studies. Accordingly sample size included 516 primiparas.

Study tools

One of the study investigators collected the sociodemographic data including

age, parity, residence, working status, educational level through face-to-face interviews with the study participants.

Eligible participants were stratified into two groups depending upon the number of attendance to ANC clinics during their pregnancy; women who came four visits or more were considered regular and those who came less than four visits were considered irregular. Recruitment included all consecutive eligible participants until the required sample size had been fulfilled.

The study tool was a structured interview questionnaire introduced by one of the researchers in the postpartum ward at the time of admission to the study. The interview was conducted face to face and the questionnaire included data about medical history, the ANC attendance, the barriers of regular ANC, the current pregnancy and labor complications, mode of delivery and neonatal outcomes.

Study outcomes

The primary outcome of the study was the difference in the rate of maternal complications during pregnancy including diabetes mellitus, hypertensive disorders, anemia and antepartum hemorrhage between both groups. The secondary outcomes were the difference in the mode of delivery, fetal birth weight, rate of neonatal complications, and need for referral to the pediatric care unit (PCU).

Statistical analysis

The collected data was coded, tabulated, and analyzed using the

statistical package for Social Science Programs (SPSS) Chicago, IL, USA, version 22. Quantitative variables were expressed as mean and standard deviation. Qualitative variables were expressed as frequency and percentage. Comparison between

groups was done using Student's T-test for quantitative variables and chi-square test for qualitative variables. Level of significance "P" value was evaluated, where P value <0.05 was considered statistically significant.

Table 1: The sociodemographic characteristics of the study participants

Sociodemographic Characteristics	Attendance of ANC				p-value
	Irregular		Regular		
	n=130	%	n=386	%	
<u>Age (years)</u>					
< 25	92	28.8			0.077
25 -<30	29	20.4	228	71.2	
30 -<35	8	20	113	79.6	
>35	1	7.1	32	80	
			13	92.9	
<u>Residence :</u>					
Urban	15	16	79	84	0.023*
Rural	115	27.3	307	72.7	
<u>Educational level:</u>					
Illiterate	27	25.7	78	74.3	0.02*
Primary	42	31.8	90	68.2	
Secondary	57	25	171	75	
University	4	7.8	47	92.2	
<u>Working status:</u>					
Worker	0	--	15	100	0.023*
House wife	130	25.9	371	74.1	
<u>Husband's education:</u>					
Illiterate	29	29.3	70	70.7	0.013*
primary	34	35.8	61	64.2	
Secondary	61	22.5	210	77.5	
University	6	11.8	45	88.2	

ANC; antenatal care, (*) statistically significant difference

Results

Six hundred and forty-four women were approached to participate in this study. One hundred twenty-eight women were excluded due to the presence of different exclusion criteria or their

refusal to participate in the study. The remaining 516 subjects were stratified into two groups according to their number of ANC visits attendance: regular; those with four or more visits (n=386, 74.8%), and irregular; those with less than four visits (n=130, 25.2%).

The sociodemographic data of the study participants are summarized in Table 1. There was a statistical significant difference between both groups regarding the residence ($p=0.023$), educational level ($p=0.02$), working status ($p=0.023$), and husband's

education ($p=0.013$). Irregular attendants tended to be less educated, living in rural area, housewives and married to less educated husbands also. On the other hand, both groups are quite similar regarding the women's age.

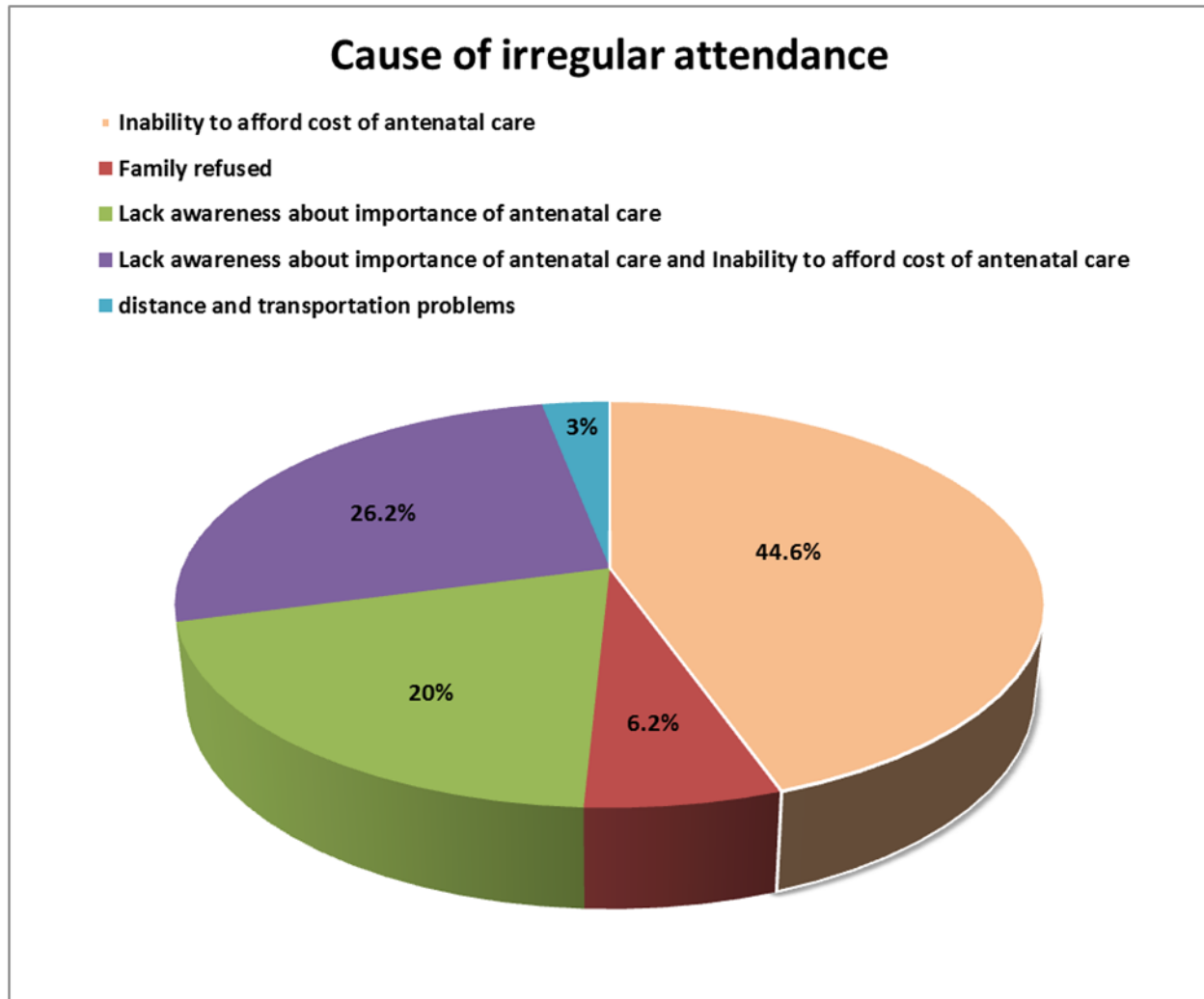


Figure 1: Causes of irregular attendance among irregular ANC group.

The percentage of women who started their ANC visits at the first-trimester was 84.1%. As regard the place of ANC, 77.3% of women preferred private clinics and Maternal and Child health centers (MCH) for attendance of ANC.

MCH centers are primary health care centers providing medical and social services for mothers and children. Figure 1 shows that either financial problems (44.6%) or lacks of awareness about importance of antenatal care

(20%) were the most common reasons for irregular attendance of ANC visits in our study. Table 2 demonstrated that the percentage of women who had had any complication in the current

pregnancy was higher in the irregular ANC group (69.2%) compared with the regular ANC group (22.8%). The difference had reached a statistically significant value ($p=0.000$).

Table 2: The maternal complications during pregnancy and labor of the study participants

Complications	Attendance of ANC				p-value
	Irregular n=130	%	Regular n=386	%	
Pregnancy induced hypertension	12	9.2	10	2.6	0.001*
Preeclampsia	48	36.9	38	9.8	0.000*
Eclampsia	12	9.2	8	2.1	0.000*
Anemia	3	2.3	1	0.3	0.021*
Oligohydramnios	13	10	18	4.7	0.027*
PROM	25	19.2	32	8.3	0.001*
Obstructed labor	3	2.3	14	3.6	0.466
Postpartum hemorrhage	0	0	2	0.5	0.411
Placenta Previa	1	0.8	5	1.3	0.628
Placental abruption	1	0.8	3	0.8	0.993
Gestational diabetes	2	1.6	5	1.3	0.192
No complications	40	30.8	298	77.2	0.000*

ANC; antenatal care, PROM; premature rupture of membranes, (*) statistically significant difference

Preeclampsia has the highest percentage in both irregular and regular ANC groups (36.9% and 9.8%, respectively), being significantly higher in irregular ANC group ($p=0.000$). Premature rupture of membranes is higher in the irregular ANC group (19.2%) compared with (8.3%) in the regular ANC group. However, gestational diabetes, placental abnormalities, obstructed labor and postpartum hemorrhage show no significant difference between both

groups.

As regard to the mode of delivery; 46 women (35.4%) in the irregular ANC group delivered vaginally, versus 149 women (38.6) in the regular ANC group with no statistical difference ($p=0.513$). Table 3 demonstrates a statistical significant difference between both groups as regard the neonatal outcome. The mean birth weight is higher in the regular ANC group ($p=0.000$). The percentage of preterm babies, stillbirths,

low birth weight babies and the rate of admission to PCU is higher in the irregular ANC group than the regular

ANC group (13% vs. 3.6%, 10% vs. 1.8%, 25.4% vs. 7.5% and 8.5% vs. 1.8%, respectively; $p=0.000$).

Table 3: The neonatal outcomes of the study participants

Outcome	Attendance of ANC				p-value
	Irregular		Regular		
	n=130	%	n=386	%	
Preterm birth	17	13	14	3.6	0.000 [*]
Stillbirth	13	10	7	1.8	0.000 [*]
Low birth weight	33	25.4	29	7.5	0.000 [*]
PCU admission	11	8.5	7	1.8	0.000 [*]
Birth weight, <i>mean ± SD</i>	2.6 ± 0.66		3.1 ± 0.65		0.000 [*]

ANC; antenatal care, PCU; pediatric care unit, SD; standard deviation,
(*) statistically significant difference

Discussion

The benefits of performing ANC have been extensively discussed in the scientific literature. The deprivation of ANC can be a contributing factor for development of preterm labor, intrauterine growth retardation, low birth weight, maternal and neonatal mortality as a result of infections in the perinatal and postnatal periods.⁸ Thus, the present study aimed to identify the factors causing lack of attendance at antenatal clinics in primiparas and to determine the effects of lack of ANC on perinatal outcomes.

Regarding the number of antenatal visits, the majority of the study sample (74.8%) had 4 antenatal visits or more and only 25.2% had 1-3 antenatal visits. These results agree with that of Pradhan et al., 2013,⁹ in Nepal who reported that the majority of the mothers

(69.5%) had completed four ANC visits. On the contrary Hawley et al., 2014,¹⁰ and Asundep et al., 2013,¹¹ reported that the highest number of respondents, (64.3%) had visited less than four times, whereas only (35.7%) had visited four times or more. the possible reason for the discrepancy could be that our study subjects were primiparas only.

Regarding the location of ANC more than half of sample (77.3%) attended ANC at a private clinic and Maternal and child health center. These results agree with Nisar & White, 2008,¹² who reported that the proportion of women seeking ANC from a private care facility (57.9%) was much higher as compared to government care facilities (14.5%). Our results disagree with that of Tuladhar & Dhakal, 2012,⁶ in Nepal who reported that most of the study participants (71.6%) had attended ANC at a hospital followed by private clinic

(20.4%).

Regarding the age and number of antenatal visits, the present study showed there was no significant difference between the different ages and number of antenatal visits. These results agree with Nisar & White¹² who reported that no association was observed when age categories were compared between regular attendants and irregular attendants. However, Abosse et al., 2010,¹³ reported that ANC service utilization is significantly influenced by maternal age. Mothers who are in the age group of 25–29 years were less likely to utilize ANC service than women who are 35 years and older. Also, Dairo & Owoyokun, 2010,¹⁴ reported that women who were 25 years or more were more likely to attend ANC clinic more than women who were less than 25 years. The explanation for the discrepancy could be due to difference in the study subjects, our study subjects were primiparous women that regardless of their ages attend antenatal care more than multiparous women.

Regarding residence and number of antenatal visits, the present study revealed that there was a significant difference between the urban and rural residents ($p=0.023$), with women residing in urban areas having more ANC visits than those residing in rural areas. These results agree with that of Ajayi & Osakinle, 2013,¹⁵ in Nigeria who reported that more urban respondents (70.1%) had four visits or more compared to rural respondents (65.8%). Also Mugo et al., 2015,¹⁶ in South Sudan reported that women living in rural areas were less likely to use ANC services, compared to their counterparts in urban areas. The possible

explanation for these results could be the distance to maternal health services and transportation problems may greatly reduce access to ANC services in rural areas.

Regarding education and number of antenatal visits, the present study showed that there was a significant difference between education and number of antenatal visits which was in line with most published results, with the majority of women who are highly educated in the regular ANC group. This could be attributed to the educated women having a higher awareness about the importance of ANC. However, Nisar & White¹² reported no association was observed when educational status was compared between the two groups.

The most frequently cited reasons for not attending ANC regularly were inability to afford the cost of antenatal care (44.6%) and ignorance about the importance of ANC (20%). These results agree Dairo & Owoyokun¹⁴ who reported that (58.3%) of women gave inability to afford the cost of ANC as the reason for not obtaining ANC at all. On the contrary, Yousuf et al., 2010,¹⁷ in Pakistan reported that (44.4%) of women did not receive ANC because the facility was far away from home and (15.8%) that the transport was not available.

Regarding the relationship between pregnancy complications and ANC, the present study revealed that 69.2% of irregular attendant mothers had pregnancy complications compared to 22.9% of regular group. The most common complication was preeclampsia among the irregular group 36.9% versus 9.8% in the regular group. These results

agree with Tuladhar & Dhakal⁶ who reported that maternal complications like anemia and pregnancy induced hypertension occurred more commonly in women without ANC. On the opposite side Kihara et al., 2015,¹⁸ in rural Kenya reported that there was no significant difference in maternal or perinatal outcomes between those who received a complete and incomplete ANC package.

There was no significant difference between modes of delivery between the two groups. These results disagree with Tuladhar & Dhakal⁶ who reported higher cesarean section (CS) rates (17.4%) in women who had attended ANC, because of the fact that all elective CS were planned in these women only. Also, all inductions of labor (9.3%) were performed among them.

The present study revealed that there was a statistical significant difference between the neonatal outcomes in regular and irregular ANC groups with far better outcomes in all aspects in the regular ANC group with ($p=0.000$). These results agree with that of Ahmed et al., 2012,² in Pakistan who reported that women with more than four antenatal visits were six times as likely to deliver normal weight babies. Also, these results agree with Raatikainen et al., 2010,¹⁹ who reported that there were significantly more low birth weight infants in under- and non-attenders, more fetal deaths and more neonatal deaths. It also agree with Tuladhar & Dhakal⁶ who reported that the proportion of low birth weight and preterm babies was higher in women with inadequate or no ANC.

The study had its strengths the large sample of the study participants included. Additionally, the target group was primigravida who are more vulnerable to pregnancy complications and are in real need for regular ANC visits. The main limitation was the possibility of recall bias as all the collected data was obtained from the women through the questionnaire in a retrospective way.

There are some confounding factors that need to be considered when interpreting the results. Even still the study presents alarming risks for irregular ANC among women in our community. We recommend performing a regression analysis model using all the women's characteristics and the reported barriers of ANC use to infer what is significantly affecting women's frequency of use.

Conclusion

In conclusion, ANC is an essential part of modern health care services. So, every pregnant woman should have full access to it. The results of this study revealed that the main reasons for inadequate utilization of ANC services were financial problems, unawareness about the importance of ANC services and family refuse. There is a significantly higher rate of pregnancy complications and adverse birth outcomes in irregular attendants. There is a need for enhancing awareness about the importance of ANC and for motivating women to utilize ANC services.

References

1. El-Zanaty F, Way AA. Egypt Demographic and Health Survey 2014. Calverton, Maryland: Ministry of Health and Population [Arab Republic of Egypt], El-Zanaty and Associates, and Macro International. <https://dhsprogram.com/pubs/pdf/PR54/PR54.pdf>
2. Ahmed Z, Khoja S, Tirmizi SS. Antenatal care and the occurrence of low birth weight delivery among women in remote mountainous region of Chitral, Pakistan. *Pak J Med Sci.* 2012;28(5):800-5. <http://pjms.com.pk/index.php/pjms/article/viewFile/2544/926>
3. Joshi C, Torvaldsen S, Hodgson R, Hayen A. Factors associated with the use and quality of antenatal care in Nepal: a population-based study using the demographic and health survey data. *BMC Pregnancy Childbirth.* 2014 Mar 3;14:94. <https://doi.org/10.1186/1471-2393-14-94> PubMed PMID: 24589139; PubMed Central PMCID: PMC3943993.
4. WHO. Integrated Management of Pregnancy and Childbirth (IMPAC). Provision of effective antenatal care. Standards for Maternal and Neonatal Care 1.6, WHO, 2006. http://www.who.int/reproductivehealth/publications/maternal_perinatal_health/effective_antenatal_care.pdf
5. Andrew EV, Pell C, Angwin A, Auwun A, Daniels J, Mueller I, Phuanukoonnon S, Pool R. Factors affecting attendance at and timing of formal antenatal care: results from a qualitative study in Madang, Papua New Guinea. *PLoS One.* 2014 May 19;9(5):e93025. <https://doi.org/10.1371/journal.pone.0093025> PubMed PMID: 24842484; PubMed Central PMCID: PMC4026245.
6. Tuladhar H, Dhakal N. Impact of antenatal care on maternal and perinatal outcome: a study at Nepal Medical College Teaching Hospital. *NJOG.* 2011 Nov-Dec;6(2):37-43. <http://dx.doi.org/10.3126/njog.v6i2.6755>
7. Titaley CR, Hunter CL, Heywood P, Dibley MJ. Why don't some women attend antenatal and postnatal care services?: a qualitative study of community members' perspectives in Garut, Sukabumi and Ciamis districts of West Java Province, Indonesia. *BMC Pregnancy Childbirth.* 2010 Oct 12;10:61. <https://doi.org/10.1186/1471-2393-10-61> PubMed PMID: 20937146; PubMed Central PMCID: PMC2964562.
8. Rosa CQ, Silveira DS, Costa JS. Factors associated with lack of prenatal care in a large municipality. *Rev Saude Publica.* 2014 Dec;48(6):977-84. <https://doi.org/10.1590/S0034-8910.2014048005283> English, Portuguese. PubMed PMID: 26039401; PubMed Central PMCID: PMC4285828.
9. Pradhan PM, Bhattarai S, Paudel IS, Gaurav K, Pokharel PK. Factors contributing to antenatal care and delivery practices in Village Development Committees of Ilam district, Nepal. *Kathmandu Univ Med J (KUMJ).* 2013 Jan-Mar;11(41):60-5. PubMed PMID: 23774416.
10. Hawley NL, Brown C, Nu'usolia O, Ah-Ching J, Muasau-Howard B, McGarvey ST. Barriers to adequate prenatal care utilization in American Samoa. *Matern Child Health J.* 2014 Dec;18(10):2284-92. <https://doi.org/10.1007/s10995-013-1368-9> PubMed PMID: 24045912; PubMed Central PMCID: PMC3959630.

11. Asundep NN, Jolly PE, Carson A, Turpin CA, Zhang K, Tameru B. Antenatal care attendance, a surrogate for pregnancy outcome? The case of Kumasi, Ghana. *Matern Child Health J.* 2014 Jul;18(5):1085-94.
<https://doi.org/10.1007/s10995-013-1338-2> PubMed PMID: 23948806; PubMed Central PMCID: PMC5034862.
12. Nisar N, White F. Factors affecting utilization of antenatal care among reproductive age group women (15-49 years) in an urban squatter settlement of Karachi. *J Pak Med Assoc.* 2003 Feb;53(2):47-53. PubMed PMID: 12705483.
13. Abosse Z, Woldie M, Ololo S. Factors influencing antenatal care service utilization in hadiya zone. *Ethiop J Health Sci.* 2010 Jul;20(2):75-82. PubMed PMID: 22434964; PubMed Central PMCID: PMC3275839.
14. Dairo MD, Owoyokun K. Factors affecting the utilization of antenatal care services in Ibadan, Nigeria. *Benin J Postgrad Med.* 2011;12(1):3-13.
<https://doi.org/10.4314/bjpm.v12i1.63387>
15. Ajayi IO, Osakinle DC. Socio demographic factors determining the adequacy of antenatal care among pregnant women visiting Ekiti State Primary Health Centers. *Online J Heal Allied Sci.* 2013Apr-Jun;12(2):4. Available at URL:
<http://www.ojhas.org/issue46/2013-2-4.html>
16. Mugo NS, Dibley MJ, Agho KE. Prevalence and risk factors for non-use of antenatal care visits: analysis of the 2010 South Sudan household survey. *BMC Pregnancy Childbirth.* 2015 Mar 26;15:68.
<https://doi.org/10.1186/s12884-015-0491-6> PubMed PMID: 25885187; PubMed Central PMCID: PMC4396873.
17. Yousuf F, Haider G, Shaikh RB. Factors for inaccessibility of antenatal care by women in Sindh. *Journal of Ayub Med Coll Abbottabad.* 2010Oct-Dec;22(4):187-89.
18. Kihara AB, Harries AD, Bissell K, Kizito W, Van Den Berg R, Mueke S, Mwangi A, Sitene JC, Gathara D, Kosgei RJ, Kiarie J, Gichangi P. Antenatal care and pregnancy outcomes in a safe motherhood health voucher system in rural Kenya, 2007-2013. *Public Health Action.* 2015 Mar 21;5(1):23-9.
<https://doi.org/10.5588/pha.14.0070> PubMed PMID: 26400598; PubMed Central PMCID: PMC4525362.
19. Raatikainen K, Heiskanen N, Heinonen S. Under-attending free antenatal care is associated with adverse pregnancy outcomes. *BMC Public Health.* 2007 Sep 27;7:268.
<https://doi.org/10.1186/1471-2458-7-268> PubMed PMID: 17900359; PubMed Central PMCID: PMC2048953.